

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (71) Applicant: BAYLOR COLLEGE OF MEDICINE [US/US]; One Baylor Plaza, Houston, TX 77030 (US).         |  | Published<br><i>With international search report</i><br><i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>   |
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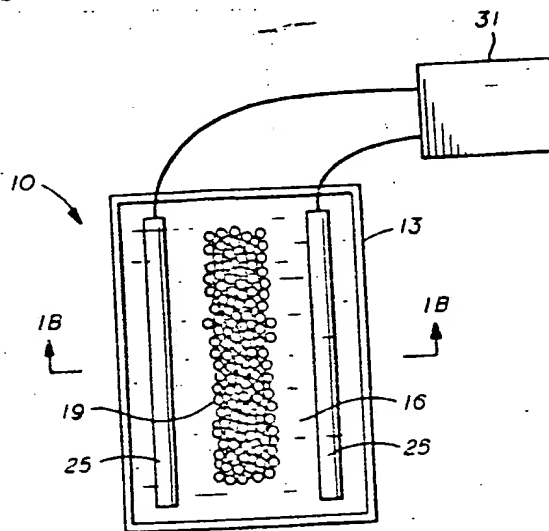
ВВЕЩЕНИЕ  
ПАТЕНТНО-СЛУЖБЫ  
БИБЛИОТЕКА

(54) Title: METHOD OF AND APPARATUS FOR CELL PORATION AND CELL FUSION USING RADIOFREQUENCY ELECTRICAL PULSES

## (57) Abstract

Disclosed are an apparatus and a method for the poration and fusion of cells using high-power radiofrequency electrical pulses. The electrodes of the apparatus can be hand held or part of integrated equipment with special containers for cells. The electrodes, which are positioned equidistant from each other, are attached to a high power function generator. The power function generator can apply a continuous AC electrical field and/or a high-power pulsed radiofrequency electrical field across the electrodes. The alternating electrical field induces cell congregation by the process of dielectrophoresis. The high-power pulsed radiofrequency electrical field porates or fuses the cells.

The method has the ability to porate or fuse biological cells with a very high efficiency. The method can be used to fuse or porate a variety of cells including animal cells, human cells, plant cells, protoplasts, erythrocyte ghosts, liposomes, vesicles, bacteria and yeasts. The method has the unique ability to porate or fuse cells in very small or very large numbers. During the poration or fusions, a variety of chemical agents including DNA, RNA, antibodies, proteins, drugs, molecular probes, hormones, growth factors, enzymes, organic chemicals and inorganic chemicals can be introduced into these cells. The method can also be used to produce new biological species, to make hybridoma cells which produce animal or human monoclonal antibodies and to insert therapeutic genes into human cells which can be transplanted back into the human body to cure genetic diseases.



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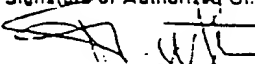
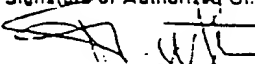
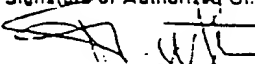
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# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03457

| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>1</sup><br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC <sup>4</sup> : C 12 N 15/00; C 12 N 13/00  |  |                                     |  |  |   |  |  |         |   |   |   |   |   |      |
|--|--|-------------------------------------|--|--|---|--|--|---------|---|---|---|---|---|------|
| <b>II. FIELDS SEARCHED</b><br>Minimum Documentation Searched <sup>7</sup><br><table border="1"> <tr> <th>Classification System <sup>1</sup></th> <th>Classification Symbols</th> </tr> <tr> <td>IPC<sup>4</sup></td> <td>C 12 N</td> </tr> </table> Documentation Searched other than Minimum Documentation<br>to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>  |  |                                     | Classification System <sup>1</sup>   | Classification Symbols   | IPC <sup>4</sup>  | C 12 N   |  |         |   |   |   |   |   |      |
| Classification System <sup>1</sup>   | Classification Symbols   |                                     |  |  |   |  |  |         |   |   |   |   |   |      |
| IPC <sup>4</sup>   | C 12 N   |                                     |  |  |   |  |  |         |   |   |   |   |   |      |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b><br><table border="1"> <tr> <th>Category <sup>9</sup></th> <th>Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th>Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td>A</td> <td>EP, A, 0128567 (KERNFORSCHUNGSANLAGE JULICH GmbH) 19 December 1984, see claims<br/>--</td> <td>1-4, 11</td> </tr> <tr> <td>A</td> <td>US, A, 4578168 (G.A. NOFMANN) 25 March 1986, see claims; column 5<br/>--</td> <td>1</td> </tr> <tr> <td>A</td> <td>US, A, 3059359 (J.H. HELLER) 25 June 1963, see claims<br/>----</td> <td>1, 5</td> </tr> </table>  |  |                                     | Category <sup>9</sup>  | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup> | Relevant to Claim No. <sup>13</sup>                         | A  | EP, A, 0128567 (KERNFORSCHUNGSANLAGE JULICH GmbH) 19 December 1984, see claims<br>-- | 1-4, 11 | A | US, A, 4578168 (G.A. NOFMANN) 25 March 1986, see claims; column 5<br>-- | 1 | A | US, A, 3059359 (J.H. HELLER) 25 June 1963, see claims<br>---- | 1, 5 |
| Category <sup>9</sup>  | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                                   | Relevant to Claim No. <sup>13</sup> |  |  |   |  |  |         |   |   |   |   |   |      |
| A  | EP, A, 0128567 (KERNFORSCHUNGSANLAGE JULICH GmbH) 19 December 1984, see claims<br>--   | 1-4, 11                             |  |  |   |  |  |         |   |   |   |   |   |      |
| A  | US, A, 4578168 (G.A. NOFMANN) 25 March 1986, see claims; column 5<br>--  | 1                                   |  |  |   |  |  |         |   |   |   |   |   |      |
| A  | US, A, 3059359 (J.H. HELLER) 25 June 1963, see claims<br>----  | 1, 5                                |  |  |   |  |  |         |   |   |   |   |   |      |
| <div style="display: flex; justify-content: space-between;"> <div> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div> |  |                                     |  |  |   |  |  |         |   |   |   |   |   |      |
| <b>IV. CERTIFICATION</b><br><table border="1"> <tr> <td>Date of the Actual Completion of the International Search<br/>30th May 1989</td> <td>Date of Mailing of this International Search Report<br/>19 JUN 1989</td> </tr> <tr> <td>International Searching Authority<br/>EUROPEAN PATENT OFFICE</td> <td>Signature of Authorized Officer<br/><br/>P.C.G. VAN DER PUTTEN</td> </tr> </table>  |  |                                     | Date of the Actual Completion of the International Search<br>30th May 1989 | Date of Mailing of this International Search Report<br>19 JUN 1989   | International Searching Authority<br>EUROPEAN PATENT OFFICE | Signature of Authorized Officer<br><br>P.C.G. VAN DER PUTTEN |  |         |   |   |   |   |   |      |
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ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.

US 8803457  
SA 25321

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| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)       | Publication<br>date  |
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| EP-A- 0128567                             | 19-12-84            | DE-A,C 3321226<br>JP-A- 60009490 | 13-12-84<br>18-01-85 |
| US-A- 4578168                             | 25-03-86            | JP-A- 61037090                   | 21-02-86             |
| US-A- 3059359                             |                     | None                             |                      |

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